Clin. exp. Immunol. (1983) **53**, 83–87.

T cell subpopulation dynamics following insulin-induced hypoglycaemia in normal subjects

H. L. BHAKRI,* H. JONES,† DAWN A. JONES,†‡ K. W. PETTINGALE* & D.E.H. TEE† **Department of Medicine and †Department of Immunology, King's College Hospital Medical School, London, UK.*

(Accepted for publication 25 February 1983)

SUMMARY

Using monoclonal antibodies OKT3, OKT4 and OKT8, T lymphocyte subpopulations were determined in eight normal male volunteers. One month later, the T cell populations were again measured before and during an insulin stress test. Compared to the month before, there was a statistically significant reduction in the numbers of OKT4 cells ($P < 0{\cdot}01$) in the basal sample. Administration of insulin produced a statistically significant rise in the numbers of total lymphocytes and in each of the T cell subpopulations at 30 and/or 60 min ($P < 0{\cdot}01$) when compared with the basal values. It was also noted that in some of the subjects, the sum of OKT4 and OKT8 cells was greater than the number of OKT3 cells after insulin administration. This suggests that under certain circumstances T cells in circulation may express both the helper and suppressor cell antigen. Insulin stress test is associated with increased production of stress hormones in response to the hypoglycaemia, and the observed lymphocyte changes may be mediated via these hormonal alterations.

Keywords T lymphocytes subpopulation hypoglycaemia stress insulin

INTRODUCTION

A number of pathological conditions have been shown to be associated with alterations in balance between mature T cell subpopulations (Bhan *et al.*, 1981; Reinherz & Schlossman, 1980). However, little is known about *in vivo* homeostatic control of these subpopulations. There is increasing evidence that the immune system may be under endocrine regulation (Anonymous, 1981). Lymphocytes have receptors for many hormones but the precise role of hormones on immune function is not known.

Now that techniques of identifying T cell subpopulations have improved it has become possible to investigate lymphocyte subpopulation changes in response to hormonal manipulation. Insulin hypoglycaemia is a standard clinical test for the investigation of hypothalamic–pituitary–adrenal function and it has already been reported that PHA lymphocyte transformation increases during this test (Iavicoli, Pozzilli & Di Mario, 1979).

In the present study we report the effect of an insulin stress test upon T cell subpopulation dynamics, in normal subjects.

‡ Present address: Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK.

Correspondence: Dr D. E. H. Tee, Department of Immunology, King's College Hospital Medical School, Bessemar Road, London SE5 8RX, UK.

MATERIALS AND METHODS

Subjects. Fasting, 9·00 am blood glucose, cortisol, growth hormone, prolactin, white cell count and lymphocyte subpopulations were measured in eight healthy male volunteers (age 19–22 years). One month later the same parameters were measured in response to an insulin stress test (0·15 units soluble insulin/kg) at 0, 30, 60 and 120 min. All subjects were instructed to abstain from alcohol and severe physical exertion for a period of 24 h prior to the experiments.

Isolation of lymphocytes. Lymphocytes were separated from heparinized venous blood by Ficoll-Hypaque (Fisons, Pharmaceutical Division) density gradient centrifugation (Böyum, 1968). The cells were then frozen (Thomson, O'Connor & Vaughan-Smith, 1974) and stored in liquid nitrogen.

Monoclonal antibodies. After checking the viability by trypan blue exclusion (90·3% ± 3·5), the T lymphocytes were characterized using monoclonal antibodies OKT3, OKT4 and OKT8 (Ortho Pharmaceutical Co., Raritan, New Jersey, USA). The characteristics of these antibodies have been defined as total peripheral T lymphocytes, helper/inducer and suppressor/cytotoxic cells respectively (Kung, Goldstein & Schlossman, 1979; Reinherz *et al.*, 1979a, 1979b, 1980).

Immunofluorescence. Aliquots of cells were incubated with respective monoclonal antibodies and then with fluorescein conjugated goat anti-mouse IgG (TCS, Slough, Buckinghamshire, UK). The fluoresceinated lymphocytes were counted using a Leitz Dialux microscope fitted with transmitted phase and incident fluorescent light.

Statistical analysis. The data were analysed using the paired Student's *t*-test.

RESULTS

The results of cell counts performed 1 month prior to the insulin stress test, termed control values, and immediately prior to the administration of insulin, termed basal values are shown in Table 1. There was a statistically significant reduction in the numbers of OKT4 cells at the basal time compared to the month before.

In response to insulin administration all subjects showed significant clinical and biochemical hypoglycaemia together with rise in prolactin, growth hormone and cortisol levels. The total

Table 1. Comparison between control and basal T-cell subpopulations

	Lymphocyte subpopulation numbers ($\times 10^6$/ml)							
	OKT3		OKT4		OKT8		T4/T8	
Subjects	Control	Basal	Contol	Basal	Control	Basal	Control	Basal
1	1·00	0·98	0·65	0·64	0·34	0·33	1·90	1·86
2	1·64	1·24	1·11	0·83	0·50	0·26	2·20	3·23
3	1·35	1·00	0·96	0·57	0·41	0·36	2·26	1·56
4	1·42	0·85	0·97	0·48	0·47	0·37	2·08	1·28
5	1·57	0·99	0·93	0·51	0·49	0·44	1·91	1·15
6	1·67	1·52	1·21	0·49	0·54	0·54	2·25	0·91
7	1·35	1·65	0·93	0·70	0·40	0·70	2·30	1·00
8	1·43	1·61	0·97	0·80	0·46	1·00	2·09	0·80
Mean ± s.d.	1·43 ± 0·21	1·23 ± 0·32	0·97 ± 0·16	0·63 ± 0·14	0·45 ± 0·06	0·50 ± 0·24	2·12 ± 0·15	1·47 ± 0·74
Paired *t*-test	N.S.		$P < 0·01$		N.S.		$P < 0·01$	

N.S. = not significant

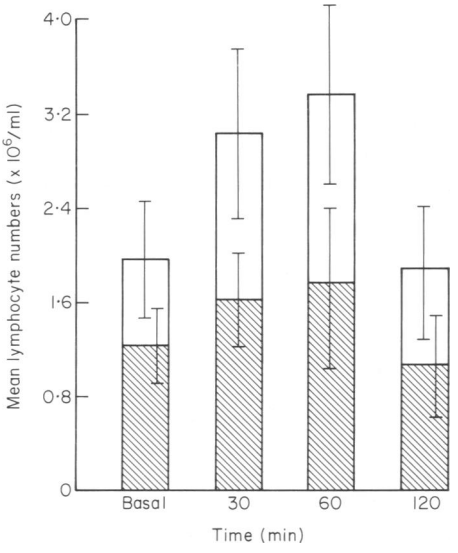

Fig. 1. Mean total lymphcyte numbers before (basal), and after insulation administration. The hatched area represents the numbers of OKT3 reactive cells. Vertical bars show ± 1 s.d.

lymphocyte count rose in each subject and reached a maximum at 30 or 60 min and then fell. The rise in OKT3 reactive cells was less than the rise of OKT3 negative cells (Fig. 1).

The insulin-induced hypoglycaemia was associated with a statistically significant rise in both OKT4 and OKT8 reactive cells at 30 and/or 60 min (Table 2). However, in six of the eight subjects the sum of OKT4 and OKT8 reactive cells after insulin administration was greater than the total number of OKT3 reactive cells. Although this difference was not statistically significant, individual differences ranged from 6–20%.

In subject No. 8, the basal OKT8 reactive cells were greater in number than the OKT4 cells and this phenomenon persisted throughout the insulin stress test.

Table 2. Helper/inducer (OKT4) and suppressor/cytotoxic (OKT8) values before and after insulin administration. The paired *t*-test compares the post-insulin with the basal sample

	Lymphocyte subpopulation numbers ($\times 10^6$/ml)							
	Basal		30 min		60 min		120 min	
Subjects	OKT4	OKT8	OKT4	OKT8	OKT4	OKT8	OKT4	OKT8
1	0·64	0·33	0·53	0·45	0·89	0·52	0·21	0·21
2	0·83	0·26	0·95	0·36	0·40	0·62	—	—
3	0·57	0·36	1·09	1·12	0·88	1·10	0·39	0·48
4	0·48	0·37	0·63	0·90	0·94	1·21	0·79	0·50
5	0·51	0·44	1·15	1·30	1·06	1·31	0·55	0·77
6	0·49	0·54	0·59	0·98	1·25	0·98	0·67	0·56
7	0·70	0·70	0·55	0·62	1·39	0·70	0·68	0·39
8	0·80	1·00	1·03	1·25	1·09	1·38	0·43	0·59
Mean ± s.d.	0·63 ± 0·14	0·50 ± 0·24	0·81 ± 0·26	0·87 ± 0·36	0·99 ± 0·30	0·98 ± 0·33	0·53 ± 0·20	0·50 ± 0·17
Paired *t*-test			N.S.	$P < 0·02$	$P < 0·05$	$P < 0·01$	N.S.	N.S.

N.S. = not significant

DISCUSSION

The ratio of helper cells (OKT4) to suppressor cells (OKT8) in normal subjects has been reported to lie within a range of 1·5–2·5 (Bach & Bach, 1981; Bhan *et al.*, 1981; Reinherz & Schlossman, 1980). Our subjects were within this range when they were tested a month prior to the insulin stress test. However, the ratio was significantly decreased on the day of the experiment in the basal sample and in some of the subjects the ratio was reversed. Alteration in OKT4:OKT8 ratio have been shown to occur in various disease states (Bhan *et al.*, 1980; Bach & Bach, 1981); but the explanation for this finding in healthy subjects is not clear. Undoubtedly, our subjects experienced varying degrees of anxiety immediately before the test and we postulate that this psychological reaction altered that ratio of helper and suppressor cell population.

The OKT3 antibody is thought to indentify 100% of peripherally circulating mature T lymphocytes (Reinherz & Schlossman, 1980). In our experiment the insulin stress test was associated with a statistically significant total lymphocytosis and an increase in the number of OKT3 reactive cells. However, the percentage of OKT3 cells actually decreased after insulin administration. By extrapolation, this means that the observed lymphocytosis represents a relatively greater increase in the number of OKT3 negative cells.

Throughout the experiment, in subject 8 the number of OKT8 cells was greater than the number of OKT4 cells. However, he had previously experienced a viral type illness and high OKT8 cell numbers have been reported after viral infections (Reinherz & Schlossman, 1980).

Peripherally circulating T cells are thought to be functionally mature and while the antigen T4 and T8 are experessed on mutually exclusive subpopulations they all express the T3 antigen (Reinherz & Schlossman, 1980). However, in some of the subjects following administration of insulin the sum of percentages of T4 and T8 became greater than the percentages of T3 cells. This would suggest either the presence of T cells which co-express both OKT4 and OKT8 antigens and/or that some of the cells did not express the T3 antigen; all such cells are known to be present in the thymus during lymphocyte differentiation and are thought to be functionally undifferentiated (Reinherz & Schlossman, 1980). Cells bearing both OKT4 and OKT8 antigen have been found to occur, in significant numbers, in the circulation in myasthenia gravis and these cells were probably of thymic origin (Berrih *et al.*, 1981).

The present experiments indicate that the total peripheral lymphocyte counts and the balance between various T cell subpopulations may alter rapidly in response to an acute stress such as insulin induced hypoglycaemia and the accompaning neuroendocrine changes. These cellular changes probably occur as a result of redistribution between the vascular compartment and the lymphoid organs. However, the immunological implication of the observed lymphocyte alterations are unknown.

We wish to thank Miss Joan Butler and Dr John Keenan, Department of Chemical Pathology for providing the hormonal data.

We are also most grateful to the medical student volunteers for their co-operation.

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